CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

d-LIMONENE

Chemical Code # 000979, Tolerance # 50304
SB 950 # 314

Original: June 26, 2003

I. DATA GAP STATUS [See comment below regarding waivers]

Chronic toxicity, rat:	No study submitted.
Chronic toxicity, dog:	No study submitted
Oncogenicity, rat:	Inadequate study, possible adverse effect indicated
Oncogenicity, mouse:	Inadequate study, no adverse effect indicated
Reproduction, rat:	No study submitted.
Teratology, rat:	No study submitted.
Teratology, rabbit:	No study submitted.
Gene mutation:	Inadequate study, no adverse effect indicated
Chromosome effects:	Inadequate study, no adverse effect indicated
DNA damage:	Inadequate study, no adverse effect indicated
Neurotoxicity:	Not required at this time

Toxicology one-liners are attached.
All record numbers through 131861 were examined.
** indicates an acceptable study.
Bold face indicates a possible adverse effect.
File name: T030626
Original: Kishiyama and Gee, 6/26/03

On October 10, 1995, the Office of Environmental Health Hazard Assessment staff concurred with waiving the SB950 mandated studies for limonene. The memorandum was addressed to Barry Cortez of the Pesticide Registration Branch by Anna Fan, Chief, Pesticide and Environmental Toxicology Section, OEHHA.
In September, 1994, the US EPA issued a "Reregistration Eligibility Decision (RED)" for Limonene.

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may contain additional effects.

COMBINED, RAT

No Study submitted

CHRONIC TOXICITY, RAT

No study submitted.

CHRONIC TOXICITY, DOG

No study submitted.

ONCOGENICITY, RAT

028 131681 "NTP Technical Report on the Toxicology and Carcinogenesis Studies of d-Limonene in F344/N Rats and B6C3F1 Mice." (C.W. Jameson, Microbiological Associates, Report No. 347, NIH Publication No. 90-2802, January 1990). d-Limonene (purity >99%) was administered by gavage at doses of 0 (corn oil), 75 or 150 mg/kg and 0, 300 or 600 mg/kg, respectively to male and female F344/N rats in 5 ml/kg. Male and female B6C3F1 mice were given doses of 0, 250, or 500 mg/kg and 0, 500 or 1000 mg/kg respectively for 103 weeks in 10 ml/kg. Doses were given 5 days per week. Each treatment group contained 50 animals per sex per group. **RATS.** Mortality was increased for high dose female rats and was lower for high dose male rats. Body weight for high dose rats were 4 to 7% lower than controls, beginning week 2 for males and week 28 for females. There were no treatment-related clinical signs. **Possible adverse effects**: incidences of tubular cell adenomas and adenocarcinomas of kidneys for male rats were significantly increased at both doses. Combined incidences were 0/50, 8/50 and 11/50 with increasing dose. The incidences of non-neoplastic lesions of the kidney (tubular cell hyperplasia, epithelial hyperplasia, mineralization of the renal papilla) for low and high dose male rats were also increased as was the severity of nephropathy. No such effects were found in female rats. The incidence of cataracts and retinal degeneration increased in a dose-related manner but was considered related to the positioning of the cages during the study, with the high dose groups being closest to the lighting without rotation. NOEL for males < 75 mg/kg/day (kidney changes) and 300 mg/kg/day for females (body weight). Food consumption was not measured. **MICE**: Body weight was reduced 5-15% for high dose female mice but there was no significant effect on male weights. There were no clinical signs related to treatment. Liver changes for high dose male mice were abnormal number of nuclei and cytomegaly. Neither effect was reported for female mice. NOEL = 250 and 500 mg/kg for male and female mice, respectively, based on liver changes in males and body weight in females at the high dose. UNACCEPTABLE (the number of dose levels inadequate; no food consumption, no hematological data). Study, however, is valid for the purpose for which it was designed. (Kishiyama and Gee, 6/20/03).

016 76653 Duplicate of 028 131681.

024 129889 Duplicate of 028 131681.

ONCOGENICITY, MOUSE

028 131681 "NTP Technical Report on the Toxicology and Carcinogenesis Studies of d-Limonene in F344/N Rats and B6C3F1 Mice." (C.W. Jameson, Microbiological Associates, Report No. 347, NIH Publication No. 90-2802, January 1990). d-Limonene (purity >99%) was administered by gavage at doses of 0 (corn oil), 75 or 150 mg/kg and 0, 300 or 600 mg/kg respectively to male and female F344/N rats in 5 ml/kg. Male and female B6C3F1 mice were given doses of 0, 250, or 500 mg/kg and 0, 500 or 1000 mg/kg respectively for 103 weeks in 10 ml/kg. Doses were given 5 days per week. Each treatment group contained 50 animals per sex per group. **RATS.** Mortality was increased for high dose female rats and was lower for high dose male rats. Body weight for high dose rats were 4 to 7% lower than controls, beginning week 2 for males and week 28 for females. There were no treatment-related clinical signs. **Possible adverse effects**: incidences of tubular cell adenomas and adenocarcinomas of kidneys for male rats were significantly increased at both doses. Combined incidences were 0/50, 8/50 and 11/50 with increasing dose. The incidences of non-neoplastic lesions of the kidney (tubular cell hyperplasia, epithelial hyperplasia, mineralization of the renal papilla) for low and high dose male rats were also increased as was the severity of nephropathy. No such effects were found in female rats. The incidence of cataracts and retinal degeneration increased in a dose-related manner but was considered related to the positioning of the cages during the study, with the high dose groups being closest to the lighting without rotation. NOEL for males < 75 mg/kg/day (kidney changes) and 300 mg/kg/day for females (body weight). Food consumption was not measured. **MICE**: Body weight was reduced 5-15% for high dose female mice but there was no significant effect on male weights. There were no clinical signs related to treatment. Liver changes for high dose male mice were abnormal number of nuclei and cytomegaly. Neither effect was reported for female mice. NOEL = 250 and 500 mg/kg for male and female mice, respectively, based on liver changes in males and body weight in females at the high dose. UNACCEPTABLE (the number of dose levels inadequate; no food consumption, no hematological data). Study, however, is valid for the purpose for which it was designed. (Kishiyama and Gee, 6/20/03).

REPRODUCTION, RAT

No study submitted.

TERATOLOGY, RAT

No study submitted. The NTP study cited a publication for rats, Tsuji, M. et al, *Oyo Yakuri* 10: 179 - 186 (1975). In addition, it cited a study in mice, Kodama, R. et al., *Oyo Yakuri* 13: 963 - 973 (1977).

TERATOLOGY, RABBIT

No study submitted. The NTP report cited a publication for rabbits, Kodama, R. et al., *Oyo Yakuri* 13: 885 - 898 (1977).

GENE MUTATION

028 131681A, "Mutagenicity of d-Limonene in *Salmonella typhimurium,*" (from Haworth, S., et

al., published in *Environ. Mutagen.* 5 (Suppl. 1): 3 - 142 (1983), SRI International). d-Limonene was tested at nine concentrations from 0.3 to 3333 µg/plate for mutagenic potential using *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 with and without metabolic activation from hamster or rat liver S9. There were triplicate plates per concentration with two trials. Limonene was toxic without S9 at 33 ug/plate and higher. Only summary data with footnotes were presented in the NTP report TR 347. Data show no increase in the number of revertants with d-Limonene in either of two trials. Positive controls were functional. UNACCEPTABLE (report is incomplete). (Kishiyama and Gee, 6/20/03).

028 131681B "Induction of TFT Resistance by d-Limonene in Mouse L5178Y Lymphoma Cells." (B. Myhr et al., publ. in *Prog. Mutat. Res.* 5: 555-568 (1985) from Litton Bionetics, Inc.). d-Limonene, at concentrations from 0.01 to 60 µl/ml without S9 Mix and at 10 to 100 µl/ml with S9 mix (using Aroclor 1254-induced F344 rat liver), was evaluated in more than one trial for a mutagenic effect on mouse lymphoma cells. Treatment time was for 4 hours at 37˚C. Mutant frequency increase with d-limonene in the presence of S9 was not dose related and did not occur in the absence of S9. Summary data only were presented as part of NTP report TR 347. UNACCEPTABLE (not a complete report). (Kishiyama and Gee, 6/20/03).

CHROMOSOME EFFECTS

028 131681D "Induction of Chromosomal Aberration in Chinese Hamster Ovary Cells by d-Limonene." (Bioassay Systems Corporation, reported in January 1990 NTP report No. 347). d-Limonene, at concentrations of 0 (DMSO), 10, 30 and 100 µg/ml without S9 Mix (8-10 hour exposure followed by 2 -3 with colcemid) and at 0, 50, 150 and 500 µg/ml with S9 Mix for (2 hour exposure followed by an additional 8 - 10 hours incubation) was evaluated for chromosomal aberration potential with Chinese hamster ovary cells. There was apparently a single culture per concentration with 100 cells per concentration evaluated for chromosomal aberrations. No increase in aberrations was reported. Positive controls were functional. Summary data only with very limited information on the conduct of the study. UNACCEPTABLE. (Summary table only). Possibly upgradeable with full report. (Kishiyama and Gee, 6/24/03).

DNA DAMAGE

028 131681C "Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by d-Limonene" (Bioassay Systems Corporation). d-Limonene (with and without S9) was tested at concentrations of 16.2, 54 and 162 µg/ml in trial 1; (without S9) at 30, 50 and 100 µg/ml in trial 2 and at 15, 30, and 50 µg/ml in trial 3 for induction of sister chromatid exchanges using Chinese hamster ovary cells. Protocol was by citation only. Cells were exposed for 2 hours with activation and for a total of 26 hours without activation. BrdU was added to cultures after the removal of the medium with S9 or after the initial 2 hours without activation. Fifty cells per concentration were scored. There was no evidence of genotoxicity in trial 1 up to 162 µg/ml. In trial two without activation, the relative SCEs per cell at 100 µg/ml was 130% of control. Trial three was negative. Overall, no adverse effect was noted. UNACCEPTABLE. (Insufficient information). (Kishiyama and Gee, 6/20/03).

NEUROTOXICITY

Not required at this time.

SUBCHRONIC STUDIES

028 131681 "NTP Technical Report on the Toxicology and Carcinogenesis Studies of d-Limonene in F344/N Rats and B6C3F□ Mice." (C.W. Jameson, Microbiological Associates, Report No. 347; NIH Publication No. 90-2802, January 1990). Sixteen day Study: Microbiological Associates - 1979. d-Limonene (purity >99%) was administered by gavage at doses of 0 (corn oil), 413, 825, 1650, 3300 or 6600 mg/kg to 5 F344/N rats and 5 B6C3F1 mice /sex/group for 12 days over a 16 day period (5 days per week). All high dose rats and 5/5 males and 3/5 female rats at 3300 mg/kg died in two days. Body weight was reduced for surviving 3300mg/kg females (8%) and 1650 mg/kg male rats (10%). All but one mouse in the 3300 and 6600 mg/kg groups died within three days. Body weights of surviving mice were not affected. No clinical signs or compound related lesions were observed for either species. No worksheet. (Kishiyama and Gee, 6/19/03).

028 131681 "NTP Technical Report on the Toxicology and Carcinogenesis Studies of d-Limonene in F344/N Rats and B6C3F□ Mice." (C.W. Jameson, Microbiological Associates, Report No. 347; NIH Publication No. 90-2802, January 1990). Range-Finding Study (13-Week Study): Microbiological Associates - 1980. d-Limonene (purity >99%) was administered by gavage at doses of 0 (corn oil), 150, 300, 600, 1200 or 2400 mg/kg to 10/sex/group F344/N rats for 13 weeks, 5 days/week in 5 ml/kg. B6C3F1 mice were given doses of 0 (corn oil), 125, 250, 500, 1000 or 2000 mg/kg for thirteen weeks, 5 days/week, 10/sex/group, in 10 ml/kg. Mortality increased at the high dose of each specie and sex. For rats, 5 males and 9 females died at 2400 mg/kg/day. There were no deaths at the lower doses. For mice, on male and 2 females died at 2000 mg/kg/day and 1 female at 500 mg/kg/day. There were other deaths due to gavage error. Body weight was reduced for male rats at 600, 1200 and 2400 mg/kg/day by 6, 12 and 23% respectively. The final body weight of the surviving female at 2400 mg/kg was 11% lower than control. The weights of females at the other doses was similar to controls. For male mice in the two highest dose groups, final weights were 10% lower than controls and for female mice, 2% lower. Clinical signs for rats included rough coats, lethargy and lacrimation at 1200 and 2400 mg/kg/day (no data). For mice, clinical signs of rough coat and decreased activity also occurred at 1000 and 2000 mg/kg/day. Lesions of the kidney (regeneration and granular casts) increased in severity for male rats at all doses with severity being dose related. In mice, one female at 2000 mg/kg had an alveolar cell adenoma. No worksheet. (Kishiyama and Gee, 6/20/03).

028 131681 "NTP Technical Report on the Toxicology and Carcinogenesis Studies of d-Limonene in F344/N Rats and B6C3F□ Mice." (C.W. Jameson, Microbiological Associates, Report No. 347; NIH Publication No. 90-2802, January 1990).A supplemental 21-Day study was performed at NIEHS after the two-year main study. d-Limonene (purity >99%) was administered by gavage at doses of 0 (corn oil), 75, 150, 300, 600, or 1200 mg/kg to 18 week-old F344/N rats (12/sex/group) for 14 days over a 21-day period. Five rats/sex/group were sacrificed 24 or 72 hours after the last dose. The left kidneys were used to determine $\alpha 2\mu$-globulin and total protein. A treatment-related increase in intracytoplasmic granules in the proximal convoluted tubules was observed for males but not for female rats. The percent increase was statistically significant and dose-related in male rats but not in females. No worksheet. (Kishiyama and Gee, 6/20/03).

SUPPLEMENTAL STUDIES

005 038048 "Mutagenicity evaluation of Plant Volatiles 1:1:1:1 Blend in the Ames *Salmonella*/microsome plate test, final report." (D. R. Jagannath, Study Director, Litton Bionetics, LBI Project No.: 20988, January 1984). Plant Volatiles 1:1:1:1 Blend (30% limonene, 40% myrcene, 15% Beta-caryophyllene and 15% Alpha-pinene) was assayed at concentrations of 0.1, 0.5, 1.0, 2.5, 5.0, 10, 25, and 50 µl/plate. Assay was run with and without metabolic activation with rat liver S9. There were duplicate plates in a single trial with a 48 hour incubation period. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 were evaluated for mutagenicity. No significant increase in revertants was reported . Supplemental study (test article was a blend of 4 plant volatiles) . (Kishiyama and Gee, 6/26/03).

005 No record number. "Evaluation of plant volatiles in the cellular immune response studies of biorational pesticides. Tier 1: serum protein determination." (G. B. Cannon, Study Director, Litton Bionetics, Inc., LBI Project No. 20995, January 1984) Plant volatiles (PV), a thick clear colorless liquid (no further identification in the report), was tested for effects on the serum protein pattern as a measure of a cellular immune response. Male and female B6C3F1 mice, 5/sex/group, were given saline, PV undiluted, 10X and 100X dilutions by ip injection, 0.5 ml/mouse, on day 0. Blood counts were obtained on day 0 and at day 14. Serum samples were subjected to electrophoresis at these same time intervals. No data were presented for blood counts (see below). All animals injected with undiluted PV died. The percentages of serum protein as α-1 globulin, α-2 globulin, β globulin and γ globulin as well as albumin were tabulated for predosing and 14 days after dosing. Values were compared with the 95^{th} confidence interval. The conclusion of the author was that there were no gross immunological abnormalities as a result of acute treatment (page 3). Incomplete report. Incomplete report. Insufficient information for an independent evaluation. Test material not limonene alone. No worksheet. (Gee, 6/26/03).

005 No record number. "Evaluation of plant volatiles in the cellular immune response studies of biorational pesticides. Tier 1: blood cell counts." (G. B Cannon, Study Director, Litton Bionetics, Inc., LBI Project No. 20995, January 1984) Plant volatiles (PV), a thick clear colorless liquid (no further identification in the report), was tested for effects on blood counts. Male and female B6C3F1 mice, 5/sex/group, were given saline, PV undiluted, 10X and 100X dilutions by ip injection, 0.5 ml/mouse, on day 0. Blood counts were obtained on day 0 and at day 14. All mice given undiluted PV liquid were dead within one day of dosing. WBC and RBC counts, hemoglobin concentration and hematocrit were determined with a Coulter S plus III counter. There were no values at variance for females. For males, the white blood cell count at 100X was 7400 compared with the 7760 cutoff (95% confidence interval) and at 10X, WBC was 6300. Other parameters were not significantly affected. The conclusion of the author was that PV was toxic to the WBC in males. Incomplete report. Insufficient information for an independent evaluation. Test article not limonene but a mixture. No worksheet. (Gee, 6/26/03)